Mengmeng Sui

Aalto University
School of Chemical
Technology

Spruce bark extract as a Sun protection agent in sunscreens

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Thesis supervisor:

Prof. Tapani Vuorinen

Thesis advisors:

M.Sc. (Tech.) Jinze Dou

Ph.D. Kavindra Kesari

ABSTRACT

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This study aimed to clarify the feasibility of utilizing spruce inner bark extract as a sun protection agent in sunscreens. Ultrasound-assisted extraction with 60 v-% ethanol was applied to isolate the extract in 25-30 % yield, that was almost independent of the temperature (45-75^{o}C) and time (5-60 min) of the treatment. However, the yield of stilbene glucosides, measured by UV absorption spectroscopy, was highest after ca. 20 min extraction. Nuclear magnetic resonance spectroscopy of the extract showed that it consisted mainly of three stilbene glucosides, astringin, isorhapontin and polydatin (piceid). The maximum overall yield of the stilbene glucosides was > 20 %. Extraction with water gave a much lower yield of the stilbene glucosides.

Sunscreens composed of a mixture of vegetable oils, surfactants (fatty acids), glycerin, water and the bark extract were prepared with the low-energy emulsification method. The performance of the sunscreens was assessed by spreading them on a photosensitive paper and observing the change in color during exposure to light. Commercial sunscreens with known SPF values (15-50) were used as references. Addition of 2-5 % of the bark extract in the sunscreen provided similar protection against UV light compared to the commercial products.

Use of the spruce inner bark extract as the sun protection agent in sunscreens appeared to be potentially feasible and should be studied further.

Keywords: Spruce bark; Stilbenes; Ultrasound-Assisted Extraction; UV-Vis; NMR

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Symbols and abbreviations

Symbols

b	Path length in cuvette (cm)
AU	Absorbance units
^{1}H	Proton
^{13}C	Carbon-13

Abbreviations

DMC	Dry matter content
DMSO	Dimethyl sulfoxide
SPF	Sun protection factor
NMR	Nuclear magnetic resonance
UVA	Ultraviolet A
UVB	Ultraviolet B
UVC	Ultraviolet C
UV-Vis	Ultraviolet visible spectroscopy

Preface

I would like to thank my supervisor Prof. Tapani Vuorinen for his excellent guidance and support. I really appreciate him for offering me such a valuable and result oriented topic for my thesis work. I also would like to thank my adviser Jinze Dou, for his great support in organizing the thesis work. I want to thank my second adviser, Dr. Kavindra Kesari for his help in review and revision of my thesis language. I would also like to thank the China Scholarship Council (CSC), who supported my stay in Finland. Moreover, I would like to thank Päivi Nieminen, Markku Nieminen and Joonas Tähtinen, who has supported my thesis with the spruce bark.

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Mengmeng Sui

1 Introduction

Norway spruce is a widely used and economically important wood species in Finnish forest industry. In 2016, 14.1 million m^3 of spruce logs were consumed in production of timber, pulp and paper, which was the second largest amount among the entire round wood assortment (Natural Resources Institute Finland, 2017).

The bark is generally used as a solid fuel for energy production or simply left in the forest after harvesting (KYLLIÄINEN & HOLMBOM, 2004). Industrial debarking of the logs, the side streams of paper and pulp industry as well as residues from harvesting together provide a notable amount of wood-derived biomass (Latva-Mäenpää, 2017). The removed spruce bark is a rich source of bioactive, phenolic compounds in Finland, where the bark constitutes 10 to 15% of the total stem weight (Fengel & Wegener, 1983). With the growing interest in plant polyphenols, spruce bark stilbenoids that exists in the root, stumps and stems is an area of interest for researchers. The stilbenoids occur as stilbene glucosides and their aglucones, i.e. the free stilbenoids. Although, spruce bark contains three main stilbene glucosides: astringin, piceid and isorhapontin, which have a similar structure with the well-studied resveratrol (Latva-Mäenpää, 2017). Interestingly, stilbene glucosides are the predominant form in spruce bark extract, which shows anti-oxidative, anti-inflammatory, anti-aging, anti-cancer and anti-UV impacts and is highly beneficial in chemical protection (Roupe et al., 2006; Rimando and Suh, 2008). The production of antibiotics from biomass wastes in paper and pulp industry has been considered more as economical and environment friendly means to minimize the odor of volatile gases as well as to reduce the yellowing phenomenon of paper. Additionally, the spruce bark stilbene glucosides could possibly be utlized as UV-protection agents in sunscreens, being seen as one of the popular commercial utilization of stilbene (Ahlnäs, 2013).

As a result of growing awareness of people on the necessity to use green materials and processes, natural renewable ingredients have gained attention especially in the cosmetics industry. For instance, 40 % of the raw materials in L’ Oreal company are renewable. The use of natural ingredients respects the first principle of green chemistry. Many substances from wood or by-products of wheat, beet as well as corn are essential in cosmetic industry. These include e.g. various polysaccharides, lipids, polyols and amino acids, star anise and fennel essential oils (Philippe et al., 2011).

Now the question is why do we actually need such sunscreens, because sunshine is extremely important for human beings, as it may enhance the synthesis of Vitamin D, impel metabolism, soothe mood, improves the treatment of skin disease and tanned. However, overexposure to the sun can be highly harmful: sunburn, skin aging, skin cancer and other similar physical damage. The sun emits UV light consisting of UVC (100 – 290 nm), UVB (290 – 320 nm) and UVA (320 –

400 nm). UVC is stopped from reaching the Earth surface by ozone layer. UVA leads to long-term chronic injury with strong permeability to skin and causes immediate damage to skin. Both UVA and UVB may lead to skin cancer. Nowadays, the renewed sunscreens enhance the protection from UVA advised by NIH (National Institutes of Health), and they are encouraged to be used in daily life.

2 Research objectives

The thesis aimed to clarify the feasibility of potential use of stilbenes from spruce bark as a sun protection agent in sunscreens. Following subtopics were set to accomplish the aim.

- To study feasible methods of extracting the stilbenes from spruce inner bark in high yield.
- To quantify the effects of the extraction on the inner bark and the composition of the extract.
- To verify and quantify the performance of the spruce bark extract as a sun protection agent in sunscreens.

3 Literature review

3.1 Spruce bark

3.1.1 Structure

Spruce stem consists of four main parts: heartwood, sapwood, vascular cambium and bark (Figure 1). Heartwood is the dead, sometimes dark and hard central part of the tree. Sapwood functions to transport the water and nutrients to the top leaves from the roots at the bottom. The vascular cambium is in the outer edge of the sapwood, which has thin active layer forming the annual rings (Chris Woodford, 2017). The living phloem and periderm constitute the bark that is the outermost part of the tree and provides the protective layer for stem and roots (Boletannery, 2014). In other words, spruce bark consists of the inner bark and the outer bark. The inner bark is a living tissue, including the living phloem, and locates in innermost area of periderm. The outermost dead tissue is called the outer bark.

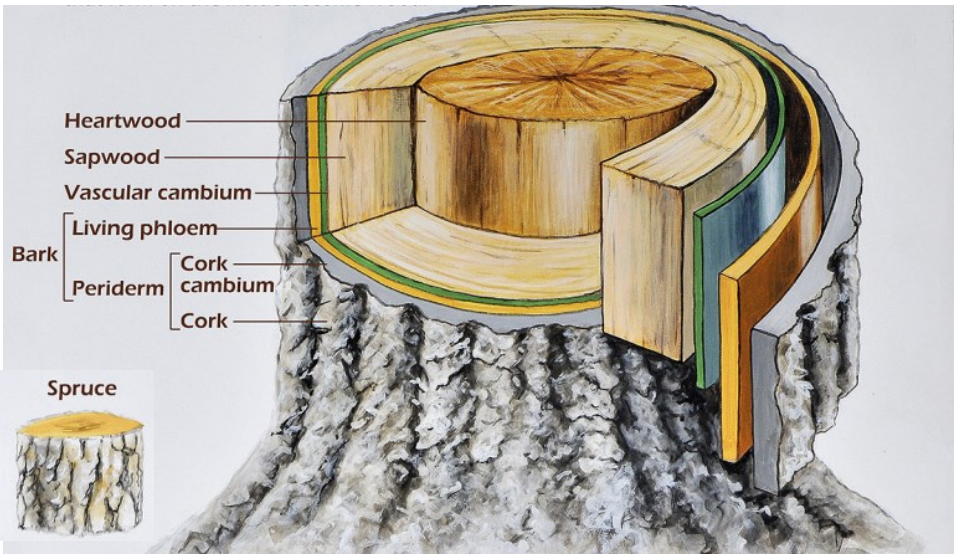

Figure 1. The structure of spruce wood (City of Loveland, 2018).

3.1.2 Chemical composition

A major difference in the chemical composition of spruce wood and bark is that the bark contains much larger amount of elements other than carbon, hydrogen and oxygen, which results from the high mineral content of the bark (Table 1) (Ugolev, 1980). The bark contains also very high amounts of extractives (Table 2). The extractive content of spruce is twice the extractive content in the branches and roots and over ten times that of spruce stem. Only the needles contain more extractives than the bark does. In contrast, the content of the main cell wall polymers, i.e.

cellulose, hemicelluloses and lignin, is relatively low in the bark of Norway spruce (Räisänen & Athanassiadis, 2013).

The extracts of spruce bark are rich in stilbenes, which occur as monomers or oligomers, often as glycosides. Most commonly monomeric stilbene glucosides, including astringin, isorhapontin and piceid, dominate over their aglycones and oligomeric stilbenes. In one case, the stilbene monomers constitute 86 % of all stilbenes while the dimers formed the remaining 14 % of the stilbenes (Gabaston et al., 2017).

Table 1. Elemental composition of spruce wood and bark (Ugolev, 1986).

	Carbon (%)	Hydrogen (%)	Oxygen (%)	Other Elements (%)
Wood	50	6	43.5	0.5
Inner bark	51.5	5.7	38.8	4
Outer bark	44.4	6.4	45.5	3.8

Table 2. Chemical composition (% on dry matter) of different morphological parts of spruce (Räisänen and Athanassiadis, 2013).

	Cellulose	Hemicellulose	Lignin	Extract
Stem wood	42 ± 1.2	27.3 ± 1.6	27.4 ± 0.7	2 ± 0.6
Bark	26.6 ± 1.3	9.2 ± 1.1	11.8 ± 0.9	32.1 ± 3.8
Branches	29	30	22.8 ± 1.7	16.4 ± 2.6
Needles	28.2	25.4	8.4 ± 2.1	43.3 ± 2.3
Stump	42.9	27.9	29.4 ± 1.8	3.8 ± 0.2
Roots	29.5	19.2	25.5	15.7

3.1.3 Utilization

Spruce logs contain 10 - 15 v-% bark, which equals to an annual bark production of 0.9 - 1.3 million tons in Finland (Anonymous, 2012). The bark has been used mainly as a solid fuel in the

forest industry. Besides, other energy uses have been considered, such as gasification and production of ethanol. Moreover, spruce bark can also be utilized in composting and gardening as bark mulch (The Middlebury Landscape, 2013). Spruce bark is also a potential raw material for adhesives, plastics as well as resins (Kemppainen et al., 2014). Moreover, the spruce bark has been also used as the agent for leather coloring (Boletannery, 2014). The bark could be used as insulation materials for heat and sound (Kain et al., 2013) and as a gas cleaning agent (Randall et al., 1976). In industry, spruce bark has been used as a preferred material in producing tannin, which covers up to 10.7 % content of wood-free bark (Surminski, 2007; Kemppainen et al., 2014). Besides this, spruce bark is also rich in bioactive compounds and polyphenolics, which provide spruce bark extract with antioxidative nature (Deng et al., 2012). Gabaston (2017) indicated spruce bark extract could be used as an anti-fungi agent toward downy mildew. Additionally, spruce bark is an important raw material in producing particle board (Blanchet et al., 2000). It can also be used to produce chip boards and panels. With the development of technology and the existing extraction techniques, spruce bark is considered highly potential as a commercially valuable biomass fraction for production of various chemical substances (Kemppainen et al., 2014 & Kemppainen, 2015).

3.2 Stilbenes

3.2.1 Structure and chemical composition

For the first time, stilbenes were determined as phytoalexins from plants in 1899, comprising an essential structural skeleton, where an ethylene bridge connects two aromatic rings, as illustrated in Figure 2. Stilbenoids are the secondary metabolites of stilbenes. Over 800 novel stilbenoids (ranging from monomers to octamers) have been isolated and identified during more than a century of study. Interestingly, more than 19 stilbenes were identified in spruce bark extract (Gabaston et al., 2017).

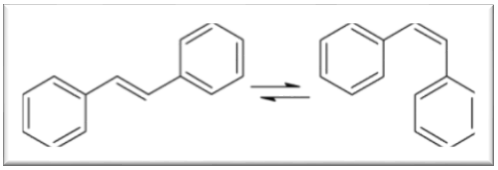

Figure 2. The trans-isomer (left) and cis-isomer (right) of stilbene (Latva-Mäenpää, 2017).

Besides lignin, stilbene glucosides are the particular phenolic compounds in spruce bark extract (Latva-Mäenpää, 2017). Astringin, isorhapontin and piceid are the main stilbene glucosides of spruce bark (Gabaston et al., 2017) (Figure 3). Their aglycones, piceatannol, isorhapontigenin and resveratrol, are less dominant and less stable compared to the stilbene glycosides. Piceatannol is the predominant free stilbenoid in spruce bark, whereas the resveratrol is the most-studied one,

representing 10 % of total amount of stilbenes (Kylliäinen & Holmbom, 2004). In nature, trees can produce stilbene aglycones specifically after a fungal attack (Viiri, 2001).

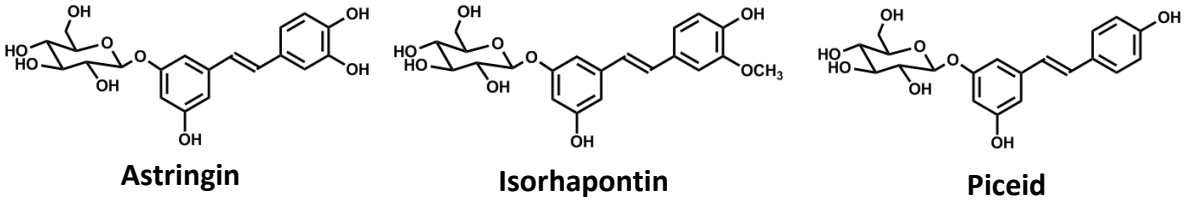

Figure 3. The structure of trans-stilbenoids from Norway spruce.

3.2.2 Seasonal and position variation

In general, bark is relatively heterogeneous in terms of morphology and chemical properties, and big differences may exist between species and even individuals of the same species. The stilbene glucosides from the bark show variation in the concentration with the season and the position (Latva-Mäenpää, 2017).

Mulat et al. (2014) reported that the isorhapontin and astringin were the main components in spruce root bark, with contents of 29.5 g/kg and 10.7 g/kg of dry mass, respectively. Besides this, Toscano & Pearce (1991) showed that spruce root bark have the highest astringin and isorhapontin contents in the month of April. On the other hand, Angelis et al. (2016) and Latva-Mäenpää (2017) reported that astringin (11.57 g/kg bark; 62.7% total stilbene) and isorhapontin (2.59 g/kg bark; 14% total stilbene) are the main stilbenoids components in spruce bark. Moreover, Jyske et al. (2015) also indicated that the higher amounts of stilbene glucosides can be gained in spruce inner bark during mid-summer to autumn and winter. Content of stilbenes may also depend on other factors like the soil and the environment (Latva-Mäenpää et al., 2017). For instance, Norway spruce typically grows in peatland and mineral soil in Finland. Moreover, seasonal fluctuation affects the aerobic and anaerobic status of peatland by changing the ground water level. The various behavior of tree root could affect the chemical composition of root bark.

3.2.3 Properties

3.2.3.1 Storage

The raw spruce bark should be stored in cold and dry place for avoiding light exposure. It has been proven that the prolonged weathering decreases the yield of extract because of leaching (Bianchi, 2017; Kelsey & Harmon, 1989). Bianchi et al. (2016) demonstrated that spruce bark extracts contain mostly phenolic oligomers after a 469-day of storage. However, there were nearly equal amounts of phenolic monomers and phenolic oligomers in fresh bark extracts. Bianchi et al. (2016) also indicated that the lower extract yields could be attributed to the longer

storage period, and the HPLC-UV spectroscopy could express the decay of phenolic monomers, which mainly exist as stilbene glucosides, i.e. astringin and isorhapontin.

3.2.3.2 Solubility

Bianchi et al. (2016) indicated that the phenolic monomers, monosaccharides and oligosaccharides are the most extractable compounds, which are solubilized in cold and warm water largely. In contrast, the extraction of tannin and polysaccharides requires an elevated temperature. Thus, a sequential extraction with an increasing temperature turned out to be an appropriately and efficiently method for recovery of polyphenolic compounds.

In general, stilbenoids belong to phenol subfamily. The compounds with a polyphenol structure have strong connection with proteins, which can easily be decomposed by acetone/ethanol-water mixture. Stilbene glucosides can be dissolved in cold water although they are more soluble in hot water. Addition of ethanol increases the solubility of stilbene glucosides up to 60% - 70% ethanol content which gives the highest extraction yield (Beijing University of Chinese Medicine, 2011).

3.2.3.3 Stability

Stilbenes exist as the *cis*- and *trans*-stereoisomers (Figure 1). *trans*-Stilbene is a white crystalline solid at room temperature. It can be easily isomerized to *cis*-stilbene under UV radiation and other conditions. The *trans*-stilbenoids occur predominantly in nature as they are thermodynamically more stable structures in comparison with the corresponding *cis*-stilbenoids (Likhtenshtein, 2009). Stilbene glucosides are unstable in water at high temperature why there is a negative relationship between their content and temperature over 80 °C (Yukun, 2009).

The naturally existing *trans*-stilbenoids may isomerize to *cis*-stilbenoids with exposure to light, and UV light can faster the isomerization process. The photochemical transformation and intramolecular cyclisation start to occur in the stilbene structure immediately after UV light exposure. The transformation is typically completed within 10 to 30 minutes of exposure. Stilbenoids may affected by pH in addition to temperature and UV light. Trela & Waterhouse (1996) found that *trans*-resveratrol may be stable for several months with proper protection from light except in high-pH buffer solution. However, *cis*-resveratrol was stable in neutral pH solution when protected from light.

Prokop et al. (2006) claimed that solid resveratrol and piceid are stable in ambient conditions independent of possible exposure to light. *cis*-Resveratrol transfers to the more thermodynamically stable *trans*-resveratrol (Trela & Waterhouse, 1996). However, piceid has been found to be less vulnerable in terms of oxidation (REGEV-SHOSHANI et al., 2003). In one

study it was claimed that sterically hindered *trans*-piceid is blocked to transfer to *cis*-piceid (Brandolini et al., 2002). Piñeiro et al. (2016) proved that stilbenoids have high stabilities under extraction conditions, the three main stilbenes in their study, i.e. piceatannol, *trans*-resveratrol and *trans*-viniferin, were recovered in over 90% yield.

3.2.3.4 Anti – UV ability

Flavonoids, other polyphenols and anthraquinones are the main anti-UV agents of plants. Plant extracts possess the biological functionality for sun protection which is based on the chemical and physical UV-screening (Nianfang, Jianqiao & Li L., 2011). The *trans*-stilbenoids have maximum UV absorption at several wavelength of 280, 306 and 320 nm (Mulat et al., 2014), and similarly *cis*-stilbenoids at 220 nm to 280 nm (Hu et al., 2008).

3.3 Extraction and isolation methods

Preferably, spruce bark is freeze-dried and milled into fine powder before solvent extraction. Thermal drying has been found one of the simplest method to get dry materials, although the yield losses are possible due to evaporation and decomposition of thermo-labile phenolic compounds at high temperature (Piñeiro et al., 2016). Prior to the extraction of stilbenoids, more lipophilic compounds need to be removed from the bark by a non-polar solvent, e.g. n-hexane, to minimize their interference (Evensen et al., 2000 & Pietarinen et al., 2006, III).

Polyphenols, like stilbenoids, conjugate easily with other components. Due to their antioxidative nature, the polyphenols are also built to react chemically with other components. Thus, rapid and efficient methods are needed to isolate the natural stilbenoids from plants. The extraction could be carried out in many ways, as summarized in Table 5. The hot water extraction, accelerated solvent extraction (ASE) and supercritical fluid extraction (SFE) are the most frequently adopted methods. However, hot water extraction has been considered to be one of the most environmental friendly method (Co et al., 2012), although it requires a long time and consumes large amount of water as the solvent. ASE and SFE both require high cost equipment. Moreover, ASE uses high temperatures and SFE requires high pressures to operate (Camel, 2000 & Cho et al., 2005). High-performance liquid chromatography (HPLC) is most frequently used for isolation of pure components and in analytical studies in combination with other methods (ultrafiltration, high-speed countercurrent extraction). HPLC is usually equipped with powerful detectors, such as Diode-Array Detector (DAD) and Nuclear Magnetic Resonance (NMR) detector (Mulat et al., 2014 & Piñeiro et al., 2016) for assessing the purity of the extract fractions. However, such HPLC systems are expensive and typically tricky in operation. HPLC consumes high standard solvents and requires extremely long separation times with small input flow rates.

Recently, macro-porous resin adsorption method has become popular in separating and purifying Chinese herb extracts. The mechanism of separation could be interpreted as differences between the components in polarity, molar mass, affinity with the resin and distribution in the solvents. Chen et al. (2015) tested five different types of macroporous resins, among which XAD-7HP showed the highest adsorption and desorption capacity leading to a fraction of anthocyanins with 93.6% purity. Using macroporous resin adsorption, Lv et al. (2008) achieved a final stilbene glucoside concentration of 819 mg/g and 99% pure acicular crystals of stilbene glucosides.

Ultrasound-Assisted Extraction (UAE) has become another popular, inexpensive and easy to operate technique, which has been utilized in isolation of herb extracts. UAE offers high production efficiency, consumes low amounts of solvents, needs low temperature and energy input, accelerates the mass transfer and enhances the extraction kinetics (Cho et al., 2006 & Chemat et al., 2008). Vinatoru (2001) also stated that ultrasound is a powerful tool in extraction field and can be easily applied at small and large scales industries. Piñeiro et al. (2016) found that UAE offered over 90% reproducibility and saved nearly 90% of extraction time. Normally, a polar organic solvent, such as ethanol, acetone and their mixtures with water, are used to extract stilbenoids from the bark. Table 3 provides more details on various extraction methods applied for spruce bark.

Table 3. The extraction methods, conditions and yields of certain spruce bark extracts with citations.

Main extracted component	Solvent and sample amount	Temperature and time	Stilbene glucosides yield	Analysis method	Citation
Stilbene	60 ml acetone with 2 g bark	6 hours in Soxhlet extraction; temperature was not mentioned	0.53% - 8.29%	GC - MS; HPLC	(Latva-Mäenpää et al., 2013)
Stilbene glucosides	80 ml acetone-water (9:1 v/v) with 26 g inner bark	100 °C for 15 min	13.3%	GC	(Krogell et al., 2012)
Stilbene glucosides and a low-molecular-mass tannin	100 ml acetone with 20 g inner bark	Room temperature overnight	1% - 6%	NMR; HPLC	(Zhang & Gellerstedt, 2008)
Stilbene glucosides	2 ml acetone-water (95:5 v/v) with 20 mg bark. Heptadecanoic acid, betulinol and *p*-coumaric acid added as internal standards (0.2 mg/ml)	Ultrasonic water bath for 30 min	2.7% - 4.8%	GC - MS	(Jyske, 2014)
Stilbene	MeOH	-	1% - 6%	RP - HPLC	(Mannila & Talvitie, 1992)
Stilbene	30 l ethanol-water (85:15 v/v) with 3 kg bark	60 °C for 2 hours	1.8%	UHPLC - MS; NMR	(Gabaston et al., 2017)

3.4 Utilization in sunscreens and health care

Michelle (2010) reported that the extracts from spruce bark (Picea abies) can be used as antioxidants. The stilbenoids have a strong UV light absorption between 220 and 340 nm, which may allow stilbenoids to protect human skin from being damaged by UVB and part of UVA light. The spruce bark extract has already been utilized in cosmetics with the growing popularity in natural cosmetics. For example, Paula's choice skin care company uses Picea excelsa wood extract as skin-smoothing agent (PAULA'S CHOICE, 2018). Lucas Meyer Cosmetics uses Picea Mariana bark extract as UV block agent (Lucas Meyer Cosmetics, 2017). Borealne® Protect contains black spruce extract rich in *trans*-resveratrol, which offers photo-aging protection ability (Lucas Meyer Cosmetics, 2017). Moreover, The Lucas Meyer Cosmetics pronounced that the spruce bark extracts show great possibility in UV protection and UV-generated radical species scavenging as well as high anti-inflammatory activity. The products offer the soothing effect and long-lasting hydration to sensitive skin with recommended extract dosage around 0.1-0.5%, which have been tested in both vitro and clinical trials (Lucas Meyer Cosmetics, 2018).

4 Materials and methods

This study aimed to find out feasibility in utilizing spruce bark extract as a sun protection agent in sunscreens. Reaching of the target was assisted with characterization of spruce inner bark and extract via chemical analyses of the extracts. All the experiments were done in triplicate.

4.1 Materials

4.1.1 Source of raw materials

A 50-year-old spruce tree was kindly provided by the farm Mantereen tila (Lempäälä, Finland). Inner bark was manually peeled from the tree on March 13, which was one week after the logging (Figure 4). The collected inner bark was stored in a cold room (ca. 5 ^{o}C) before further processing. DMSO-*d6* and pyridine-*d5* used as solvents for NMR spectroscopy were purchased from Merck. Resveratrol was obtained from Evolva SA, and the plant oils and wax were bought from Limepop (Helsinki, Finland). All the other chemicals were purchased from Sigma-Aldrich.

Figure 4. Spruce inner bark collection at Mantereen tila.

4.1.2 Raw material preparation

Firstly, the spruce inner bark was cut into pieces, freeze-dried and then milled into a powder of < 1 mm particle size. The lipophilic components were removed from the powder by a 15 min treatment with n-hexane at 75 ^{o}C. Thereafter, the n-hexane extracted powder was collected, dried in oven at 40 ^{o}C for overnight and stored in cold room for further processing (Figure 5). The dry matter content of each sample was measured according to SCAN-C 39:97 standard method (Table 4).

Figure 5. The collected raw spruce inner bark (left) and n-hexane treated bark powder (right).

4.2 Methods

An overall procedure for the extraction, identification, quantification and analysis of stilbenes from the spruce inner bark is shown in Figure 6. The n-hexane treated bark powder was extracted with water or aqueous ethanol assisted with ultrasound. The extract was oven-dried and analyzed by UV-Vis and NMR spectroscopy. The overall chemical composition of the bark and the extract was analyzed according to standard methods listed in Table 4. Sunscreens were finally produced with the tested extract.

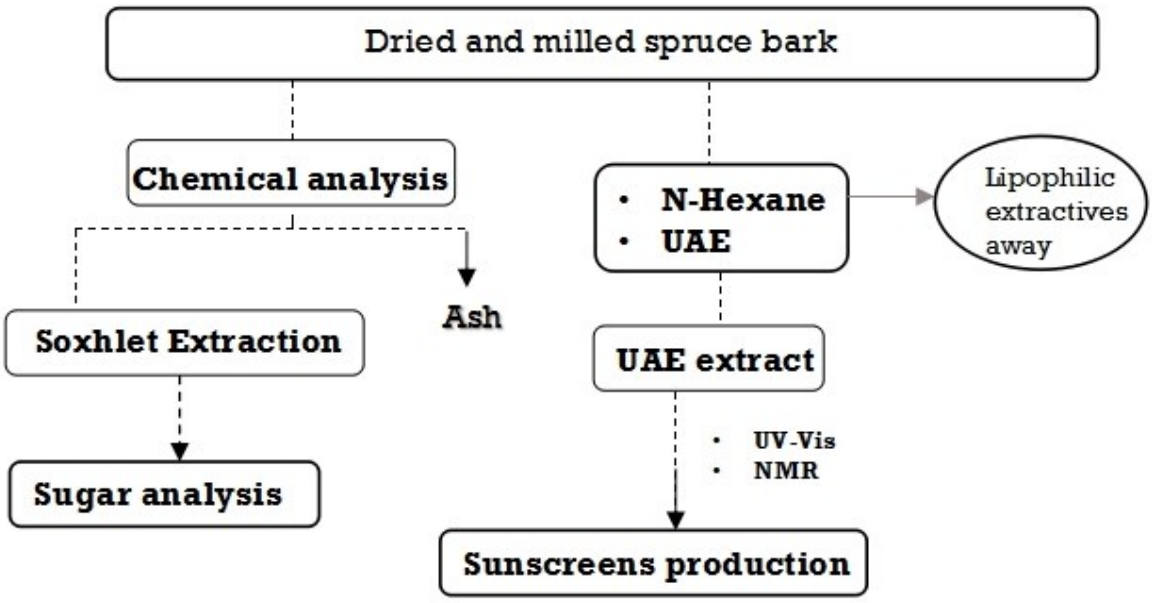

Figure 6. The process diagram for the extractions and chemical characterization of the bark and the extracts.

Table 4. Standard methods used in chemical analyses.

Analysis	Standard
Dry matter content	SCAN-C 39:97
Soxhlet extraction	SCAN-CM 49:03
Ash content	NREL/ TP 510-42622
Lignin and carbohydrate content	NREL/TP 510-42618

4.2.1 Ultrasound-Assisted Extraction

The extraction was done alternatively in 60 v-% ethanol and deionized water using an ultrasound assisted system (ultrasonic cleaner USC 600 TH). The ultrasound-assisted extraction was carried out by varying temperature (45, 60 and 75 °C) and time (from 5 to 60 min). The solid-to-solvent ratio was 1:30. All the extraction experiments were conducted in the dark using aluminum film for protection from light. The extracts were filtered by crucibles (pore size 16-40 µm) and oven-dried at 40 °C overnight. Pictures from the different steps of the extraction procedure are shown in Figure 7.

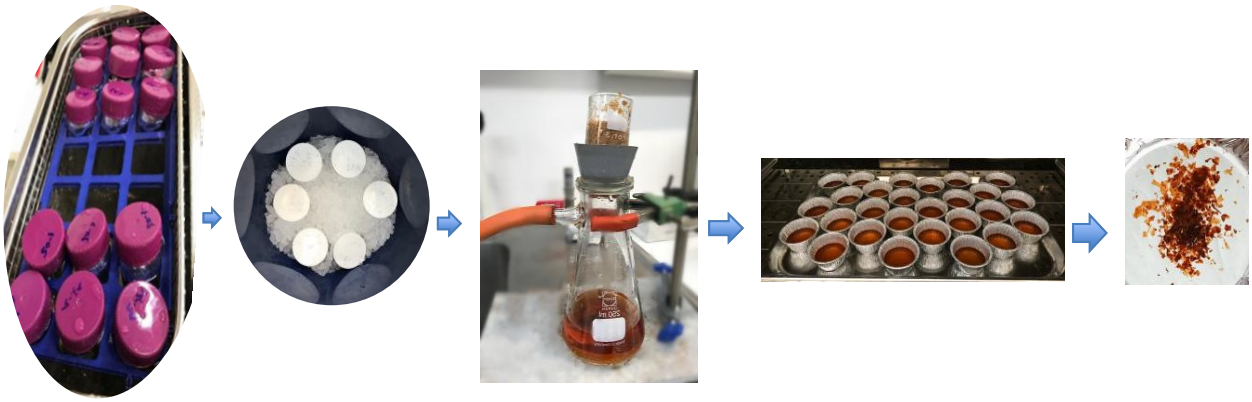

Figure 7. The ordered pictures of Ultrasound-Assisted Extraction procedure.

4.2.2 Analytical techniques

4.2.2.1 UV-Vis spectroscopy

Ultraviolet-visible (UV-Vis) absorption spectroscopy is a simple, convenient and routinely used characterization method for the quantitative determination of known chemical substances in solution. In this case, the content of stilbenoids was estimated from the UV absorption intensities by applying an external standard for calibration of the method. In general, *trans*-stilbenes have a UV absorption maximum near 295 nm while *cis*-stilbenes have two absorption maxima near 225 and 270 nm. UV-Vis absorption spectra of *trans* and *cis* isomers of resveratrol are shown in Figure 8, with absorption maxima at around 310 and 280 nm, respectively.

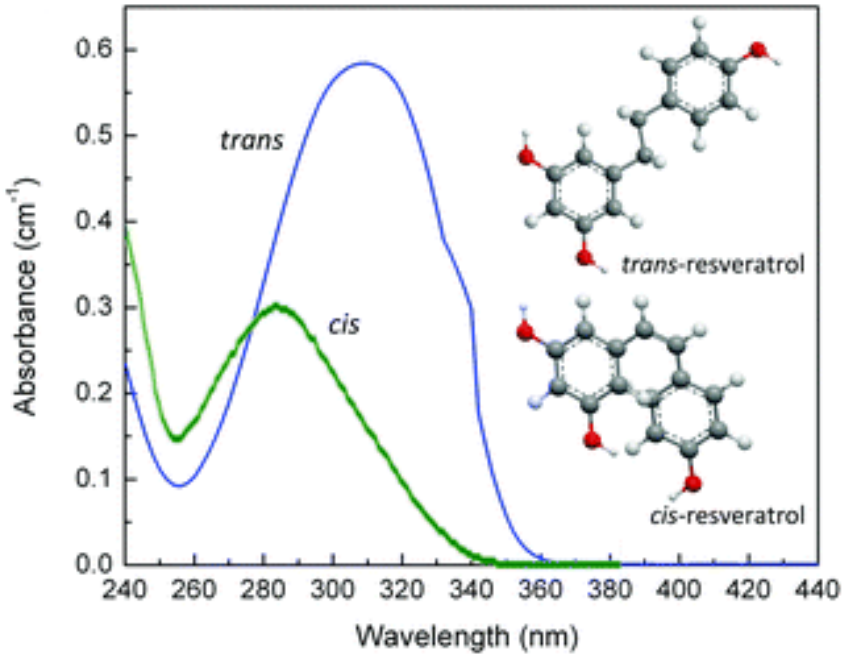

Figure 8. the UV-Vis absorption spectra of trans- and cis-resveratrol (Silva et al., 2013).

A UV-Vis absorption spectrophotometer (Shimadzu UV-2550, T1040-1002) with 10 mm quartz cuvettes was used for measuring the UV absorption spectra (190 – 400 nm) of the extracts and the external standard (Figure 9). All the samples were diluted to UV absorbance range of 0.2 – 0.7 AU (absorbance units) that is the optimal linear range of the instrument (Anonymous, 2018). Due to the lack of authentic samples of all of the major stilbenoids, *trans*-piceid (polydatin) was used as an external standard for calculating the stilbene yields from the extracts (Wrolstad, 2001).

The calibration curve (line) was constructed from the absorbances of 1.6, 4, 6.2, 7.6, 10 and 11.6 μg/ml polydatin at 320 nm.

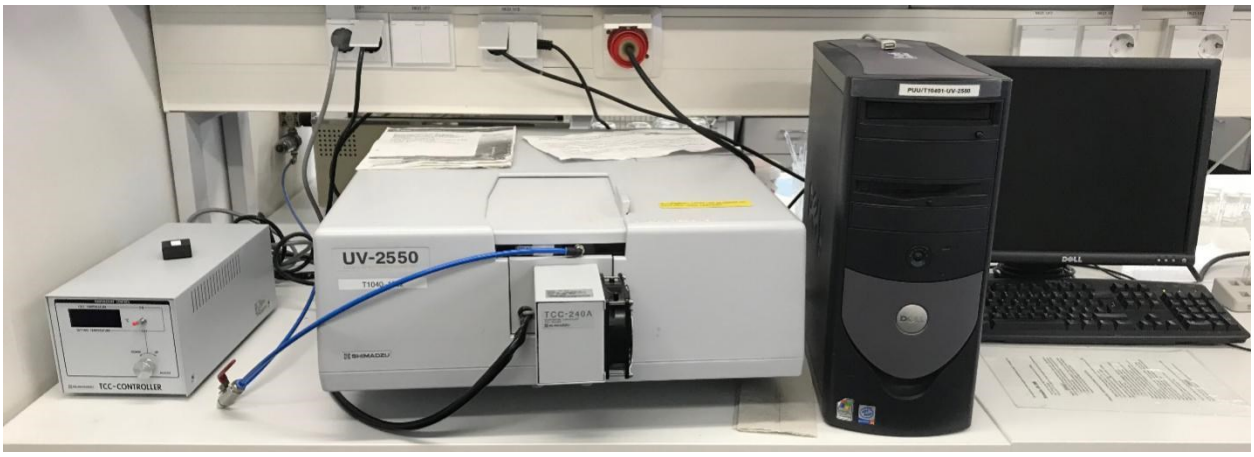

Figure 9. Shimadzu UV-2550 UV-Vis spectrophotometer.

4.2.2.2 Nuclear Magnetic Resonance spectroscopy

The nuclear magnetic resonance (NMR) spectroscopy, one of the most popular analytical tools, observes specific quantum mechanical magnetic properties of atomic nuclei, typically ^{1}H and ^{13}C, and provides structural information of the detected compounds through the chemical shifts and coupling constants of the nuclei. In this study, NMR spectroscopy was opted to determine the presence and quantity of stilbenoids in the spruce inner bark extract. The ^{1}H and ^{13}C chemical shifts of the stilbenoids were extracted from 2D HSQC and 1D ^{1}H and ^{13} C NMR spectra.

The NMR spectra (Figure 10) were measured from solutions of the extracts in dimethyl sulfoxide (DMSO) –d_6/pyridine-d_5 (4:1) at 27 ^{o}C using a Bruker Avance III spectrometer operating at 400.13 and 100.61 MHz for ^{1}H and ^{13}C, respectively. The specific parameters used for ^{1}H NMR, ^{13}C NMR and 2D HSQC were adopted from Dou et al. (2018). Additionally, ^{1}H NMR spectroscopy was also applied as an absolute method of quantification (Bharti & Roy, 2012).

Figure 10. Bruker Avance III NMR spectrometer.

4.2.2.3 Chemical composition

Analysis of the overall chemical composition of lignocellulose consists of determination of ash, extractives, carbohydrates and lignin. These were tested in duplicate following the standard methods published by Sluiter et al. in 2008 (Table 4).

Extractives

The extractives were isolated from spruce inner bark with Soxhlet extraction with acetone following the standard SCAN-CM 49:03 (2003). The samples were raw spruce inner bark, lipophilic-free n-hexane extracted inner bark and the same powder after UAE (Figure 11). Four grams of each sample were extracted with 220 ml acetone for ca. one hour. A large portion of the acetone was first recycled and then collected after the extraction. The residual acetone with the extractives were transferred into weighted aluminum pots, and they were oven-dried at 40 ^{o}C for two hours. The extracted inner bark samples were also dried in oven at 40 ^{o}C for overnight. All the samples were stored in a freezer until the carbohydrate and lignin analyses. Moreover, the dry matter content (DMC) was determined at the same time with the specific analyses.

Figure 11. Soxhlet extraction with acetone: n-hexane treated spruce inner bark (left); raw spruce inner bark (middle); UAE extracted spruce inner bark (right).

Carbohydrates

The acetone extracted samples (ca. 300 mg) were treated with sulfuric acid following the widely used analytical procedure (Sluiter et al., 2008). In this treatment, the polysaccharides were hydrolyzed into monosaccharides that were then quantified by high-pressure anion exchange chromatography (HPAEC).

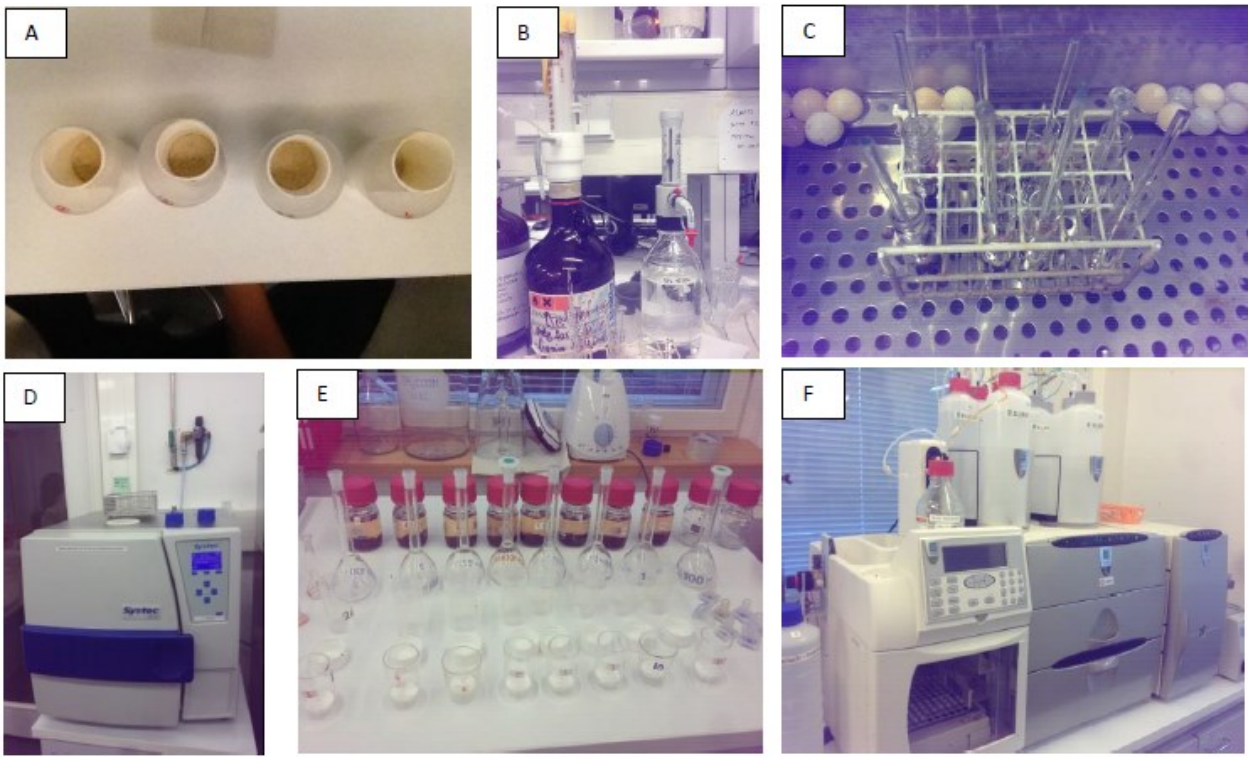

Figure 12. Steps for carbohydrate analysis: (A) spruce bark powder, (B,C) sulphuric acid and water bath, (D) autoclave, (E) sample dilution and crucible filtration, (F) high-pressure anion exchange chromatography (HPAEC) (Dou, 2015).

Lignin

Acid soluble lignin

The acid soluble lignin in the crucible-filtered hydrolyzates was determined by UV-Vis absorption spectroscopy at 205 nm.

$$Soluble\ Lignin\ (\%) = \frac{A(100 - e)}{KmD} - B$$

Where:

- ✓ A: absorbance
- ✓ e: extractive content, %
- ✓ K: absorption factor for reference lignin, K=128 l/g (softwood and softwood pulp)

- ✓ m: dry weight of the extracted sample, g
- ✓ D: dilution factor (assigned as fraction, where the numerator is the amount of sample solution and denominator is the amount of solution after the dilution)
- ✓ B: carbohydrate correction factor = 0.3 %

Insoluble Klason lignin

The insoluble Klason lignin was determined as the solid hydrolysis residue, which was oven-dried at 105 °C overnight. Then the dried residue was cooled down to room temperature in desiccator before weighing. The brushed crucibles were burnt at 550 °C for 4 h to clean them for further utilization.

$$Grav.lignin.\% = \frac{W.(100 - e)}{m}$$

Where,

- ✓ W: the weight of the hydrolysis residue, g
- ✓ e: extractive content, %
- ✓ M: dry weight of the extracted sample, g

The total lignin content was the sum of the contents of the acid soluble lignin and the insoluble Klason lignin.

Ash

The freeze-dried spruce inner bark powder was used for ash content determination. The powder was added into preweighted (± 0.1 mg) crucibles which were kept in a muffle-oven at 575 °C for 4 h (Figure 13). The ash content was calculated from the increase in the weight of the crucible after cooling down in a desiccator for 45 min (Gustafsson & Njenga, 1988).

Figure 13. Ash content determination: Muffle-oven (left) and inner bark powder before (middle) and after (right) the incubation in the furnace.

4.2.3 Sunscreen preparation

4.2.3.1 Raw material selection

Normally, chemicals (e.g. cinnamic acid ester, salicylate, and benzophenone derivatives) or physical sun protection agents (e.g. titanium dioxide and zinc oxide) compose the main UV blocking function of sunscreens (Yang et al., 2015). These popular sunscreens may cause skin irritation, or they can be hard to apply on skin. Recently, extracts from natural plants have gained increasing attention. The natural extracts may not only have the anti-UV ability, but also function as whiting and anti-aging agents, in addition to other favorable abilities (Jenab et al., 2006 & Peng et al., 2014).

Plant oils have been considered as raw materials in the production of natural cosmetics because of the favorable herbal properties, e.g. increase in the absorption capacity and softening factor of cells, inhibition of water evaporation of epidermal cells through hydrophobic film formation and protection of skin from irritation and inflammation. An oil with a higher iodine value contains more unsaturated fatty acids, which makes it easily oxidized and rotten. Different species of plant oils were selected as the oil phase materials, and the selection of raw materials was based on their physical and chemical properties, such as saponification, acid and iodine value, melting point and density. The high amount of oil affects the safety and stability of products, thus choosing proper oil is essential. The oil phases considered in this study included sunflower seed oil, apricot oil, sweet almond oil, avocado oil, aloe vera oil, olive oil and jojoba oil (Qiu & Gao, 2015).

Emulsifiers were the core elements in determining the formulation of the sunscreen: either O/W or W/O cream type. Low concentration of emulsifiers was needed to avoid elution because of de-emulsification. Adding small amount of compound oil emulsifiers could enable sunscreens de-emulsify only under higher temperature (Qiu & Gao, 2015). In this study, the emulsifiers

consisted of palmitic and stearic acids, which have commonly been used as surfactants in commercial products.

4.2.3.2 Production

The cosmetics were mostly prepared under intermittent production following the low-energy emulsification method (Figure 14). In brief, the oils and part of the aqueous phase were heated in a water bath at 70 - 80 °C before mixing them to form a concentrated emulsion. The selection of the heating temperature mostly depends on the melting point of the raw materials (Qiu & Gao, 2015; Tang & Liu, 2003). The rest of the aqueous phase, including the spruce bark extract, was then added without heating it prior to the final mixing. This sequential addition of the components rapidly cooled down the system to ambient conditions with fast agitation (Qiu & Gao, 2015; Tang & Liu, 2003). The low-energy emulsification method improves the emulsion efficiency, reduces production time and saves unnecessary waste of thermal energy (Tang & Liu, 2003).

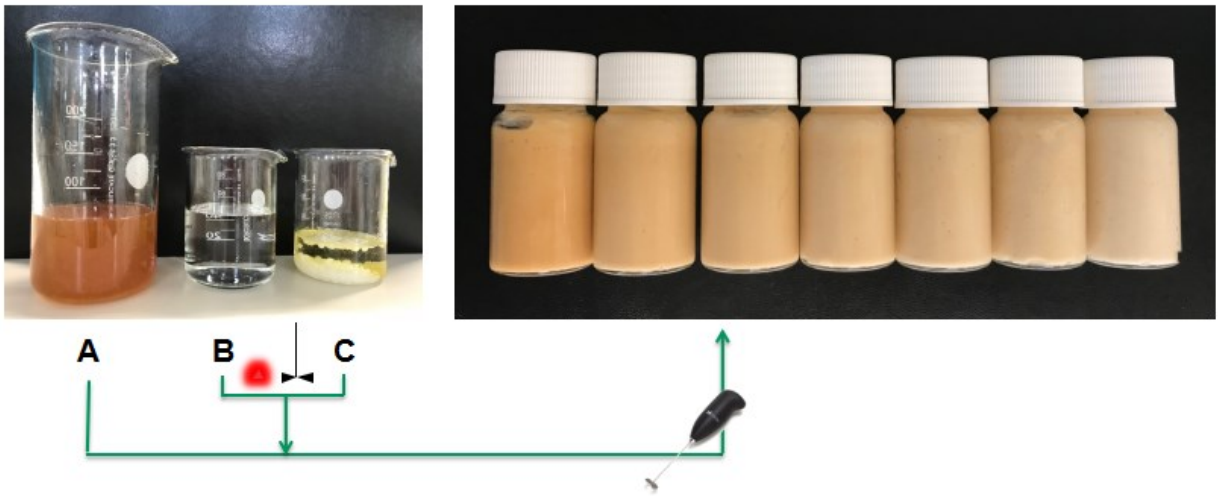

Figure 14. Preparation of the sunscreen according to the low-energy emulsification method. Aqueous phase B and oil phase C were first heated to 70 - 80 °C, then mixed to form a concentrated emulsion into which cold aqueous phase A was then mixed. The spruce bark extract was present in aqueous phase A.

Table 5. The product ingredients of sunscreens.

	Composition	Content v-%
Water phase	Deionized water	58 - 52
	Glycerin	12
	Spruce inner bark extract	2 - 8
Oil Phase	Sunflower seed oil	3
	Apricot oil	3
	Sweet almond oil	4
	Avocado oil	3
	Aloe vera oil	2
	Olive oil	2
	Jojoba oil	5
	Wax	4
(Surfactant)	Palmitic acid	1
(Surfactant)	Stearic acid	1

4.2.3.3 Properties

Various aspects were considered in the design of desired sun protection products: comprehensive protection towards UVA and UVB; good resistance to water and sweat; affinity and film formation on skin and stability of the product.

In this work, plant oils and fatty acids were used as the oil phase and emulsifiers, with HLB values around 7 and 12, respectively (Table 5). The hydrophilic-lipophilic balance (HLB) is generally applicable to non-ionic molecules and polyethylene glycerol (PEG) derivatives. In contrast, most of the plant oils have possess some ionic character and the synthetic PEG derivatives cannot be used in natural and organic cosmetic products. Moreover, every single ingredient has its specific HLB value to be emulsified, while, most of the emulsifier suppliers do not specify the exact HLB values. Thus, the HLB system was not applicable and considered in this research (Elham, 2016).

4.2.3.4 Sun protection test

The sun protection products are classified mainly into four categories depending on their SPF values and according to the legislation on cosmetics by Committee of Ministers (Table 6). The selection of appropriate sunscreens depends on the type of skin and hair, sensitivity to sun light and environmental exposure. People gets sunburn from 5 to 45 minutes after exposures to sunlight without any protection.

Table 6. The classification of sun protection ability of sunscreens in terms of UVB blocking (Skincarefoundation, 2012).

Level	SPF value
Low protection	SPFc 3 or 4
Medium protection	SPFc7, 10 or 12
High protection	SPFc 15, 20 or 25
Very high protection	SPFc 30 or 30+

The photosensitive papers were made applying aqueous solutions of ferric ammonium citrate (0.25 g/ml) and potassium ferricyanide (0.10 g/ml). These solutions were mixed together, and painting paper was soaked into the solution for 30 min. The paper was then dried in a dark room and packed in common plastic bags (Figure 15) (Alternative Photography, 2010).

Figure 15. Photosensitive papers packed in plastic.

A teaspoon of sunscreen (equal amount) was applied eventually on a single photosensitive paper of regular size and left under sunlight for 5 mins. Right after the exposure, the paper was washed with chilled water and he variation in color was compared between the samples of different sunscreens.

5 Results and discussion

5.1 Chemical composition of inner bark

Figure 16 shows the chemical composition of spruce inner bark after different treatments: original spruce bark (without treatment), n-hexane treated spruce bark, and n-hexane + UAE treated spruce bark. The carbohydrates, lignin, extractives and ash were determined experimentally, and the difference between 100 % and the total amount of the analyzed components was referred to as 'others'. These unanalyzed components include e.g. acetyl groups in hemicelluloses and uronic acids from pectins and hemicelluloses. Hardly any difference was observed between the chemical composition of the original and n-hexane treated spruce bark. Thus, the pretreatment of the spruce bark powder with n-hexane could be eliminated in the future. The UAE extracted bark powder contained negligible amounts of extractives compared to their content in the original and n-hexane treated bark. Due to removal of the extractives, the contents of carbohydrates, lignin and ash were increased in the solid residue.

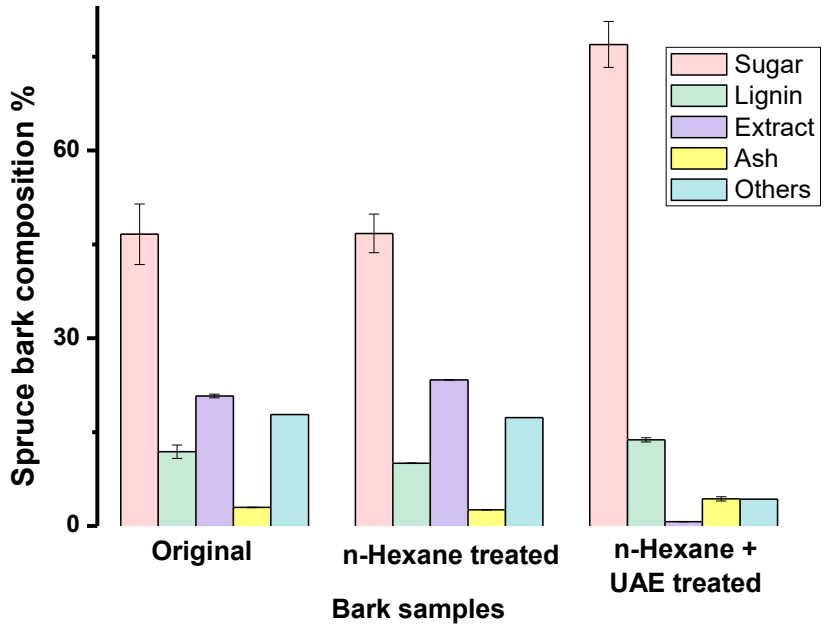

Figure 16. Chemical composition (% on original dry mass) of spruce inner bark before and after extraction with n-hexane and 60 v-% ethanol (UAE).

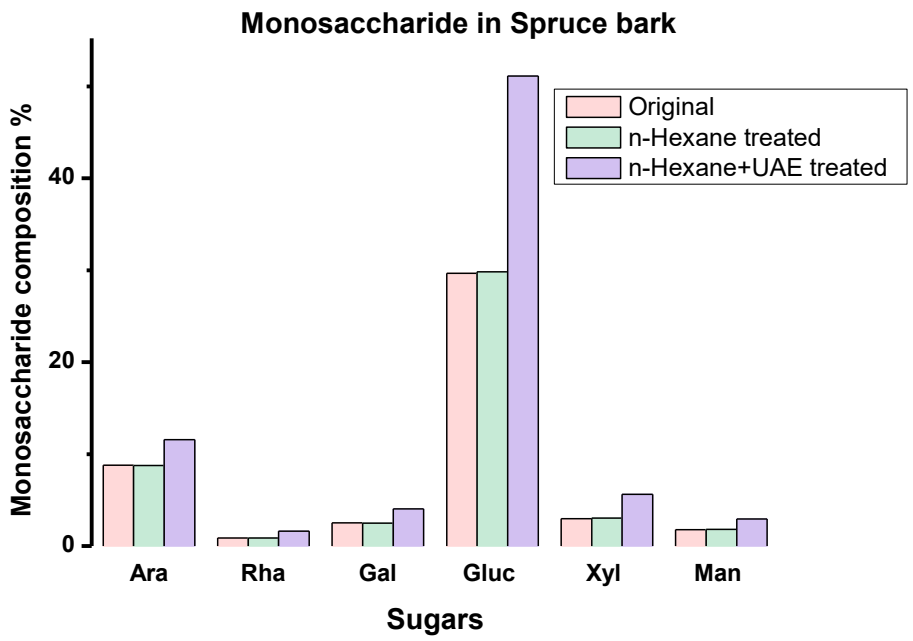

Figure 17. Monosaccharide composition (% of anhydrosugars on the original dry mass) of spruce inner bark before and after extraction with n-hexane and 60 v-% ethanol (UAE). Arabinose (Ara), Rhamnose (Rha), Galactose (Gal), Glucose (Glc), Xylose (Xyl) and Mannose (Man).

UAE increased relatively more the contents of glucose and xylose over arabinose and rhamnose in the solid residue (Figure 17). Interestingly, only traces of fructose, glucose and mannose were identified in the UAE extract (Figure 18). Acid hydrolysis of the extract released significant amounts of glucose, probably mainly from the stilbene glucosides. Moreover, typical pectic sugars, galactose, rhamnose and arabinose were released in large amounts (Vietor et al., 1995; Fang et al., 2015 & Dou, 2018). Thus, the changes in the composition of the extracted bark and of the extract both indicate that UAE partially solubilized pectic substances. It is also quite remarkable how much UAE lowered the content of 'unidentified' substances of the bark. According to the results from HPAEC, fructose was not detected in hydrolyzed extract, which include galacturonic acid of pectin. The complete disappearance of fructose in the extract after the acid hydrolysis could be explained by its conversion to hydroxymethylfurfural (HMF) under the acid conditions (Yang et al., 2011).

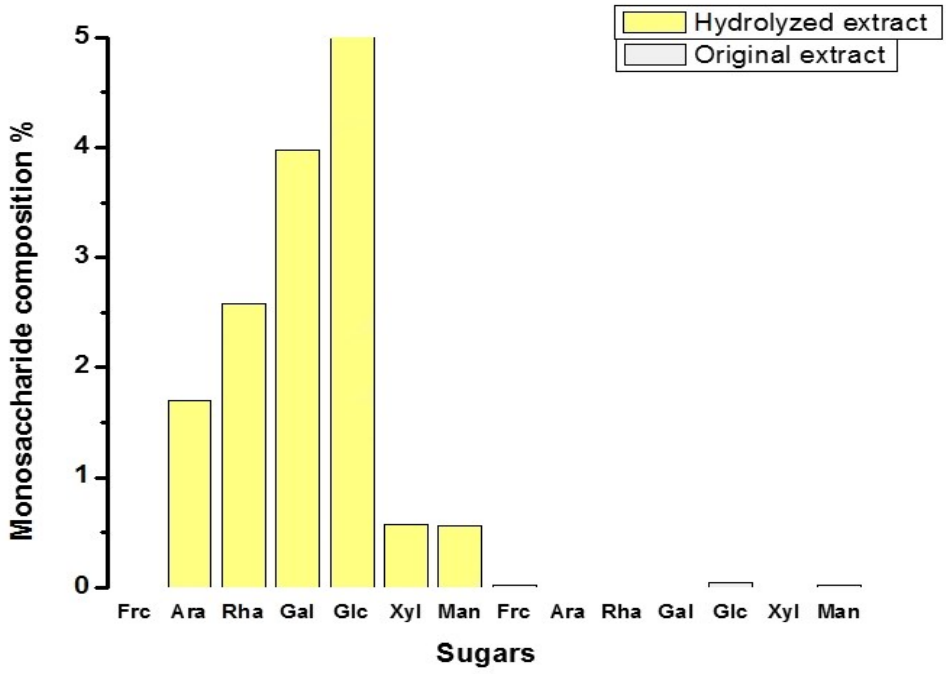

Figure 18. The monosaccharide contents of UAE extract before (right) and after (left) its treatment with acid. Arabinose (Ara), Rhamnose (Rha), Galactose (Gal), Glucose (Glc), Xylose (Xyl), Mannose (Man) and Fructose (Fru).

5.2 Quantification of stilbenes by UV-Vis absorption spectroscopy

5.2.1 Calibration curve

UV-vis absorption spectroscopy was applied in this study for rapid and non-destructive quantification of the polyphenolic components, especially stilbenes, in the extracts of spruce bark (Martelo-Vidal & Vázquez, 2014). The absorbance spectra of polydatin solutions of various concentrations are plotted in Figure 19. The shape of the spectra is typical for trans-stilbenoids, such as polydatin, with an absorption maximum at ca. 320 nm. Figure 20 represents the dependence between the absorption intensity at this maximum and concentration of polydatin which was then fitted to form a linear calibration curve for quantification of stilbenoids in the UAE extracts. The method can give only approximate values for the overall stilbene content as individual stilbenes/stilbenoids may have different absorptivities at 320 nm.

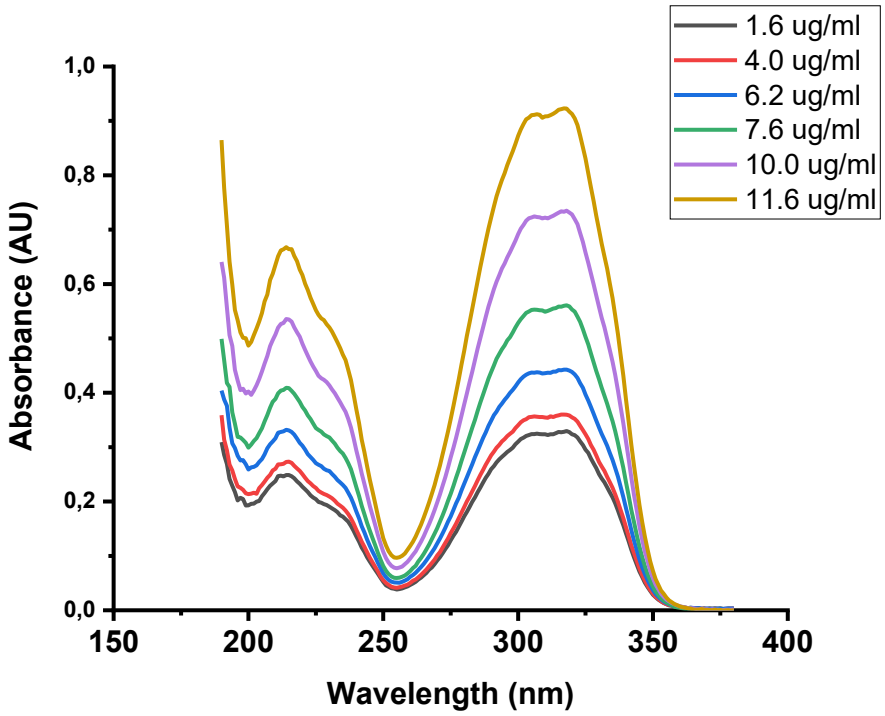

Figure 19. The UV-Vis spectra of polydatin solutions of various concentrations: 1.6, 4, 6.2, 7.6, 10 and 11.6 µg/ml.

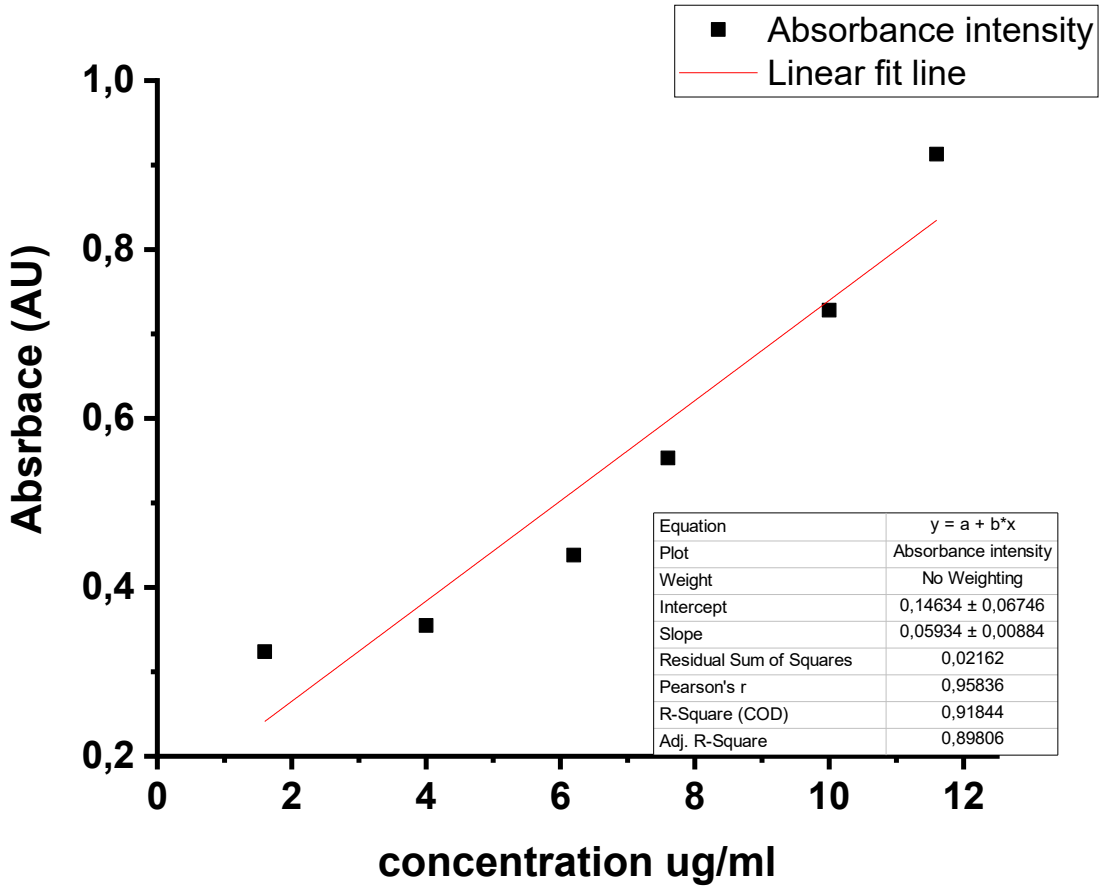

Figure 20. Calibration curve for quantification of polydatin based on its absorption intensity at 320 nm.

5.2.2 Yield of extract and stilbenes

The yields of extracts (E) and stilbenes (S) from ultrasound-assisted extraction are shown in Figure 21. It shows the yields of extract and stilbene under various temperatures (45, 60 and 75 ^{o}C) and extraction times (5, 10, 15, 20, 25, 30, 45 and 60 min). The yield of extracts was calculated gravimetrically after ultrasound-assisted extraction and oven drying of the extract. The yield of stilbenes was determined by UV-Vis absorption spectroscopy using the calibration curve of Figure 20.

Taking into account the experimental error, the extraction temperature and time hardly affected the overall extraction yield. In contrast, the stilbene yield seemed to be highest after ca. 20 – 25 min treatment irrespective of the applied temperature. The sample extracted for 20 min at the mildest conditions (45 ^{o}C) was undertaken for further analysis.

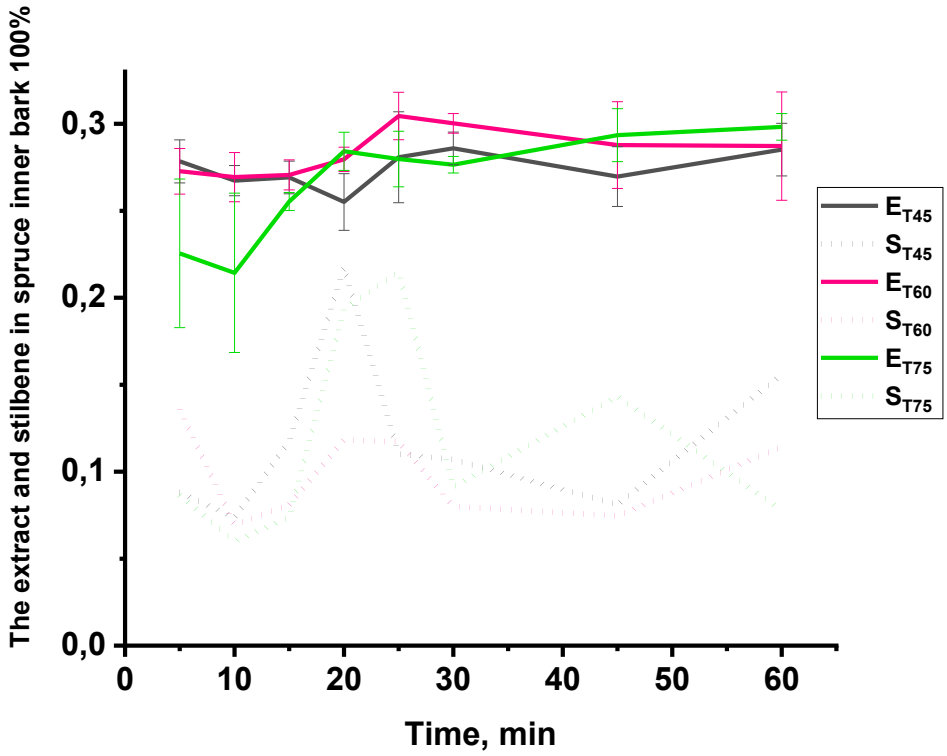

Figure 21. The yield of extracts and stilbenes from spruce inner bark under different UAE extraction temperatures and times: the yield of extracts is represented with E and solid line; the yield of stilbenes is denoted with S and dotted line. The subscript of the symbols marks the extraction temperature. The solvent was 60 v-% ethanol.

5.2.3 Stability

The stability of the selected extract (60 v-% EtOH, 45 °C, 20 min) was tested by using UV-Vis spectroscopy. The significant drop in UV absorbance at 320 nm indicated degradation of the extract after 6 weeks (Figure 22), which might be explained by conversion of the *trans*-stilbenoids into the *cis*-isomers and their subsequent oxidation (Latva-Mäenpää, 2017). Absorption of UV light can promote the isomerization (Likhtenshtein, 2009 & Trela and Waterhouse, 1996). After exposure to UV radiation, a highly fluoresecent compound was formed from *trans*-resveratrol (Yang, 2012). In 2006, Pineiro et al. & Hanzlíková et al. reported that the resveratrol is an extremely photosensitive component, which may easily undergo oxidative degradation thus forming a barrier for the utilization of resveratrol.

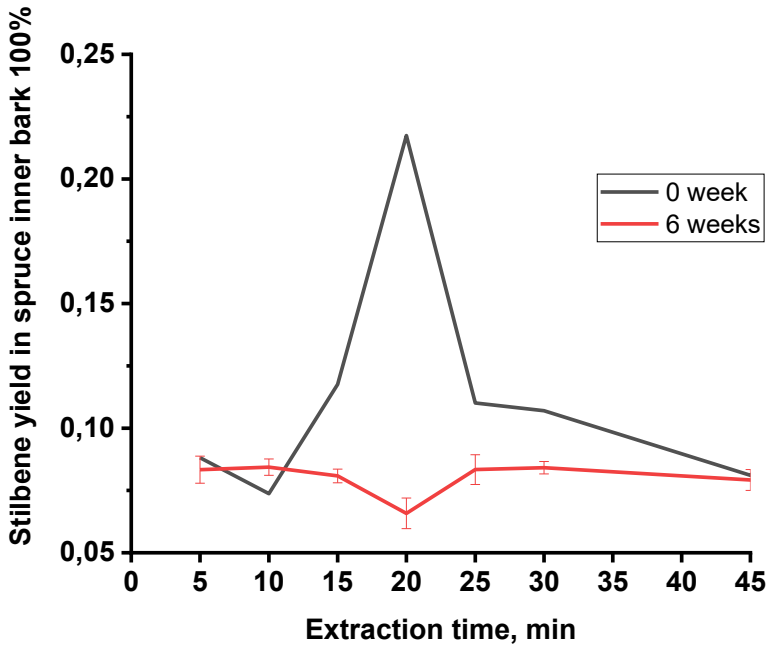

Figure 22. Stilbene yield of UAE extract (60 v-% ethanol, 45 °C, 20 min) right after the extraction 0 and after 6 weeks storage.

5.2.4 Solvent comparison

The UAE extractions were done with two different solvents: deionized water and 60 v-% ethanol. Figure 24 shows that, in comparison with water, 60 v-% ethanol provided a higher overall extraction yield and a significantly higher stilbene yield.

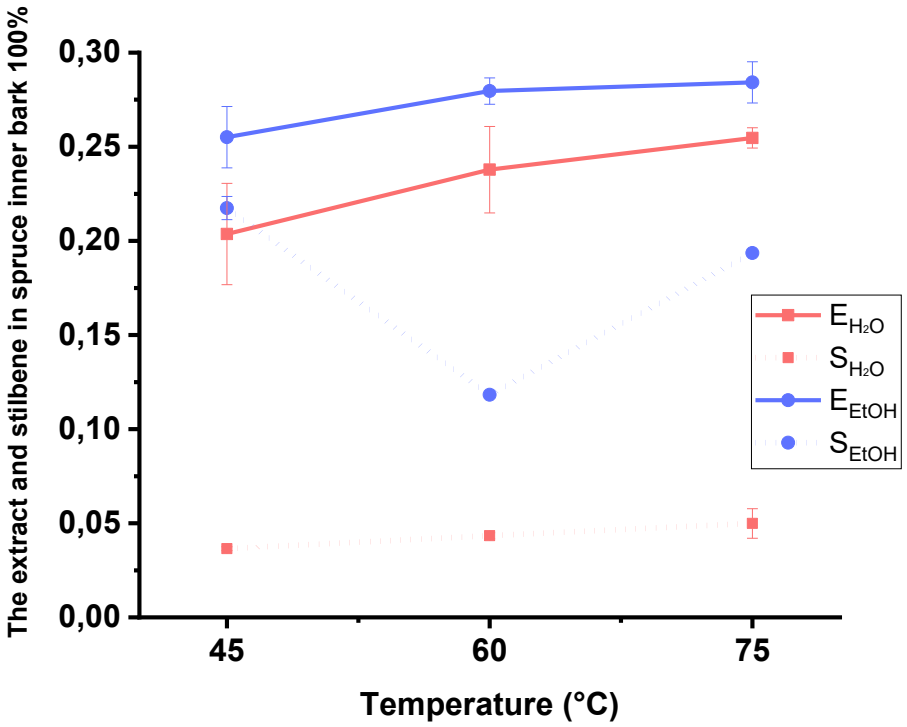

Figure 23. The effect of the solvent, 60 v-% ethanol and water, on the overall UAE extraction yield (E) and on the stilbene(S) yield at 45, 60 and 75 °C. The extraction time was 20 min.

5.3 Quantification by NMR spectroscopy

The measurements by different NMR techniques, ^{1}H NMR, ^{13}C NMR and 2D HSQC spectroscopy, confirmed that the spruce bark extracts contained stilbenes. In these spectra, polydatin, isorhapontin and astringin were labeled in different colors to better show their presence (Figures 24-26). The ^{1}H NMR, ^{13}C NMR and 2D HSQC signals of these stilbenes were assigned according to Multia (2018) and Mulat et al. (2014) who reported the spectra of the authentic compounds. The assignments are summarized in Table 7 and 8 (in appendices). In short, the spectra showed the presence of both aromatic structures and carbohydrates, confirming the presence of stilbene glucosides.

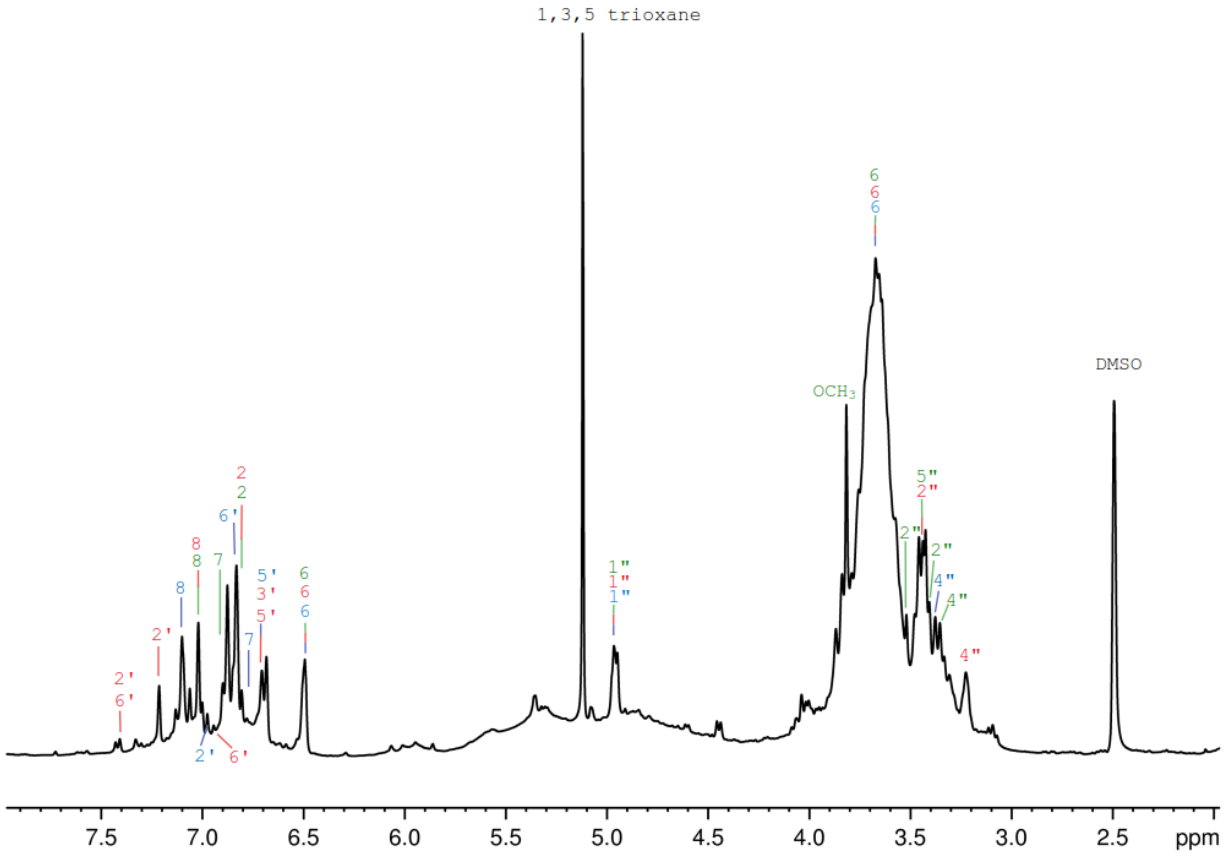

Figure 24. Assignment of polydatin (red), isorhapontin (green) and astringin (blue) in ^{1}H NMR spectrum of UAE extract of spruce bark (60 v-% ethanol, 45 °C, 20 min).

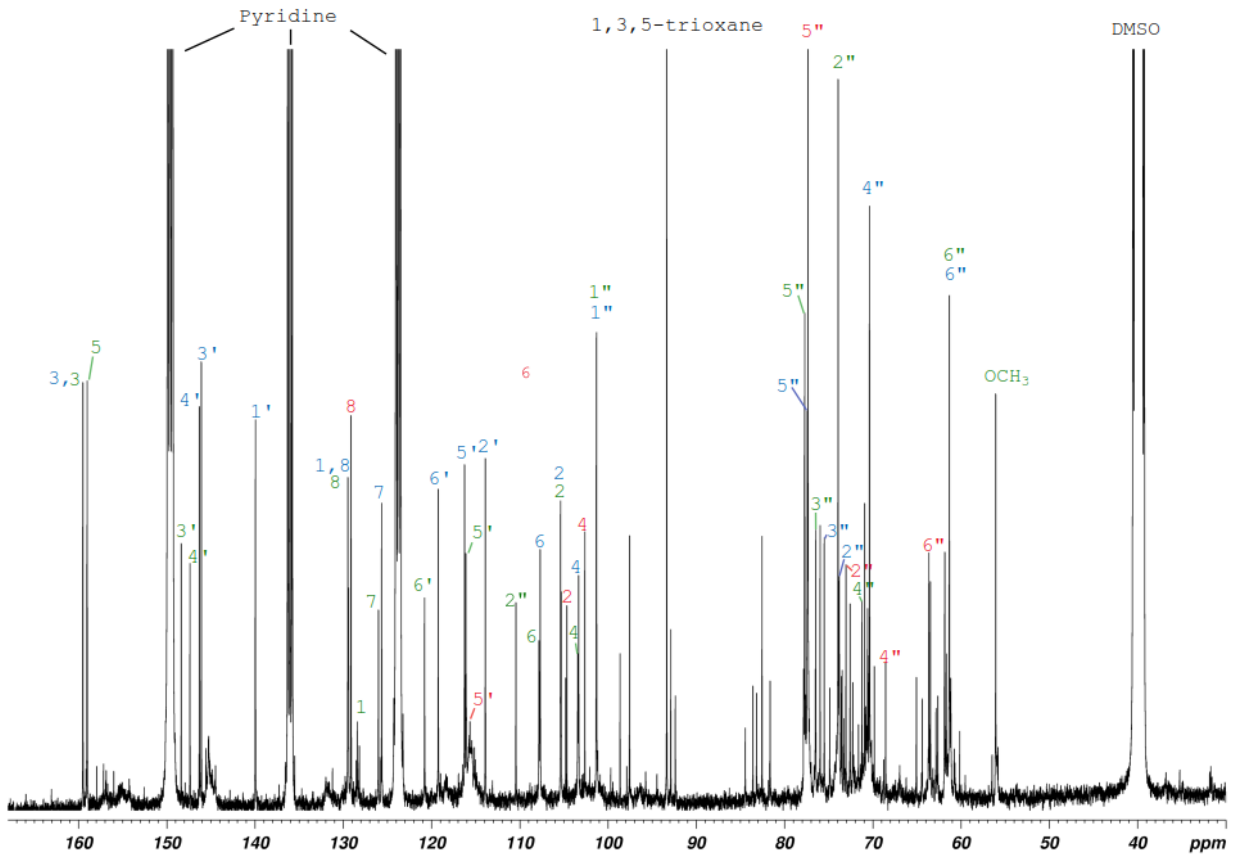

Figure 25. Assignment of polydatin (red), isorhapontin (green) and astringin (blue) in ^{13}C NMR spectrum of UAE extract (60 v-% ethanol, 45 °C, 20 min) of spruce bark.

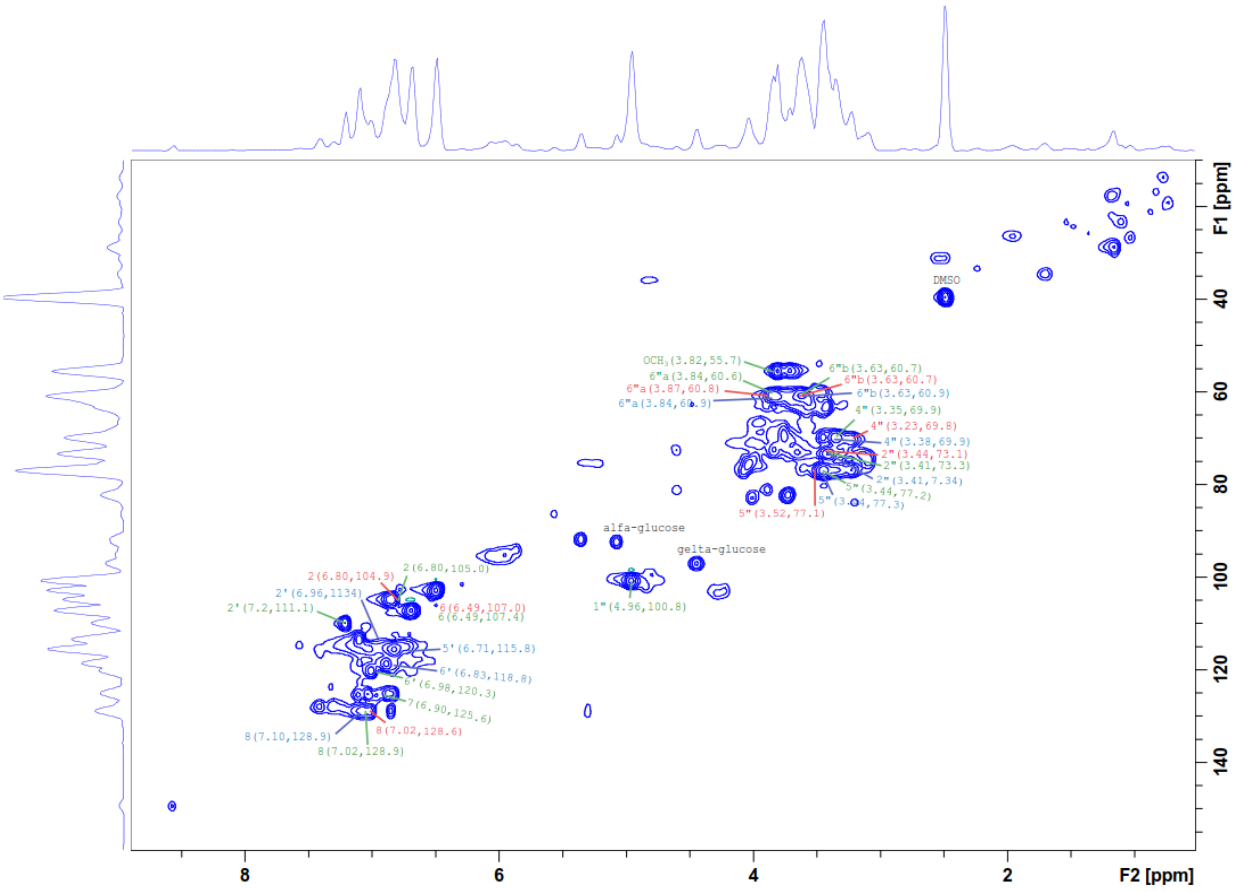

Figure 26. Assignment of polydatin (red), isorhapontin (green) and astringin (blue) in 2D ^{1}H-^{13}C HSQC NMR spectrum of UAE extract (60 v-% ethanol, 45 °C, 20 min) of spruce bark.

5.4 Sun protection ability

The UV protection ability of the spruce bark UAE extract (60 v-% ethanol, 45 °C, 20 min) in the sunscreens was tested with a photosensitive (Figure 27). Retention of light green color is indicative for good protection for UV radiation. For comparison, commercially products with SPF values of 15, 30 and 50 were also tested. The UAE extract provided a similar protection with 2-5 % additions in the sunscreen.

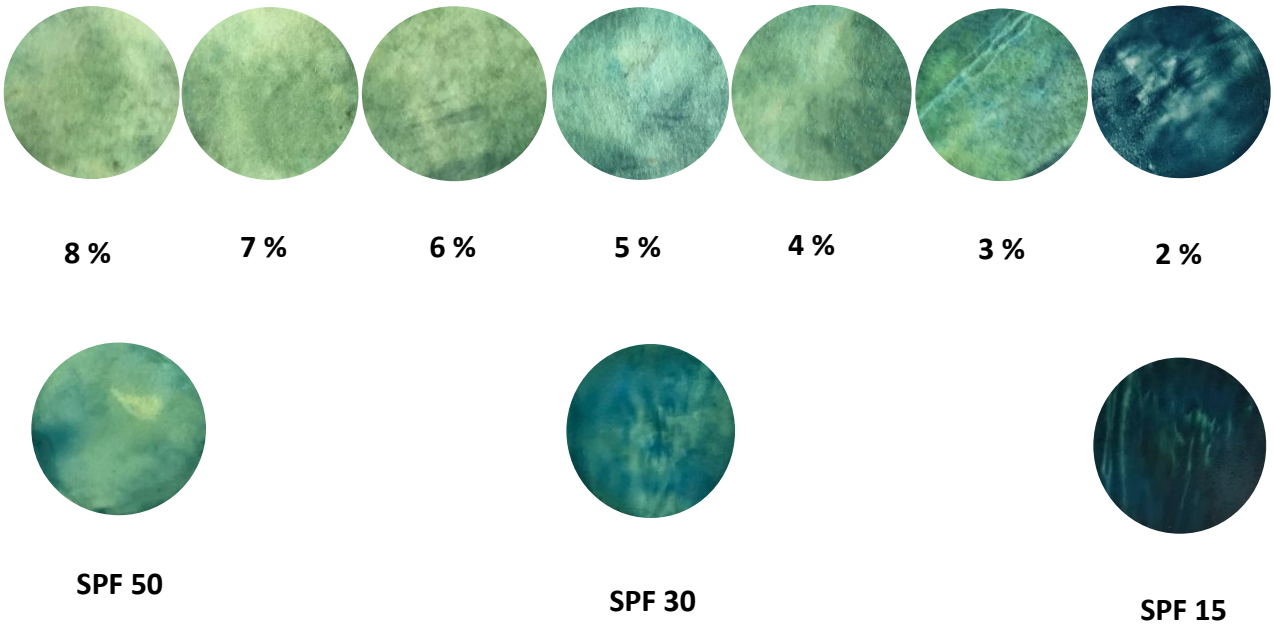

Figure 27. UV protection ability test of sunscreens with photosensitive paper. A light color is indicative for good protection. The top row shows the effect of the addition (2-8 %) of spruce bark UAE extract (60 v-% ethanol, 45 °C, 20 min) on the protection. The bottom row shows similar test results for commercial products: of SPF values of 15 (Coppertone Sport), 30 (Garnier ambre solaier) and 50 (ECO).

5.4.1 Stability

The visual stability of the sunscreens was tested after 0 and 3 weeks from their preparation. The content of the UAE extract in these samples ranged from 2 to 8 %. The separation of the oil and water phases were not observed in these formula, except the occurrence of some air bubbles (Figure 28).

Figure 28. Photos of the sunscreens after 0 week (left) and 3 weeks (right) from their preparation. The content of the UAE extract of the sunscreens is 2, 4, 6, 7 and 8% from left to right.

5.4.2 Cost

The estimated cost of the raw materials used in sunscreens production is shown in Table 11 (Appendix). The estimated cost of making the sunscreens was ca. 0.07 €/g, which excludes the cost of spruce bark extract and deionized water.

6 Conclusions

The present study concludes that ultrasound assisted extraction with 60 v-% ethanol could be an efficient and green method for isolating stilbene glucosides from spruce inner bark. Very mild conditions, 45°C and 20 min, are needed to complete the extraction. According to UV absorption spectroscopy, the yield of the stilbene glucosides can be > 20 %. NMR spectroscopy confirmed the high purity of the extract (> 90 %). Extraction with water leads to significantly lower yields of the stilbene glucosides. Use of low temperatures and dark conditions are recommended not to degrade these valuable components during the processing. The spruce bark extracts are very useful in blocking of the UV light that falls directly on the human skins. This was confirmed by using photosensitive papers, which showed a strong inhibitory effect against UV light with 2-5 % addition of the extract to the sunscreen.

7 Future research topics

From the present study, it is clear that the bark stilbenes are photosensitive compounds, which may limit their utilization in various products and materials for long-time UV protection. At this point, it would be important to explore how to enhance the stability of stilbenes e.g. through their chemical modification. Stilbenes are potentially strong to blockers of UV radiation in the range of 200-350 nm. Obviously, they should be used with other substances to provide full protection for both UVA and UVB radiation up to 400 nm that is essential for sunscreens. The emulsification process in the production of the sunscreen should be modified to prevent formation of air bubbles in the product.

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Appendix

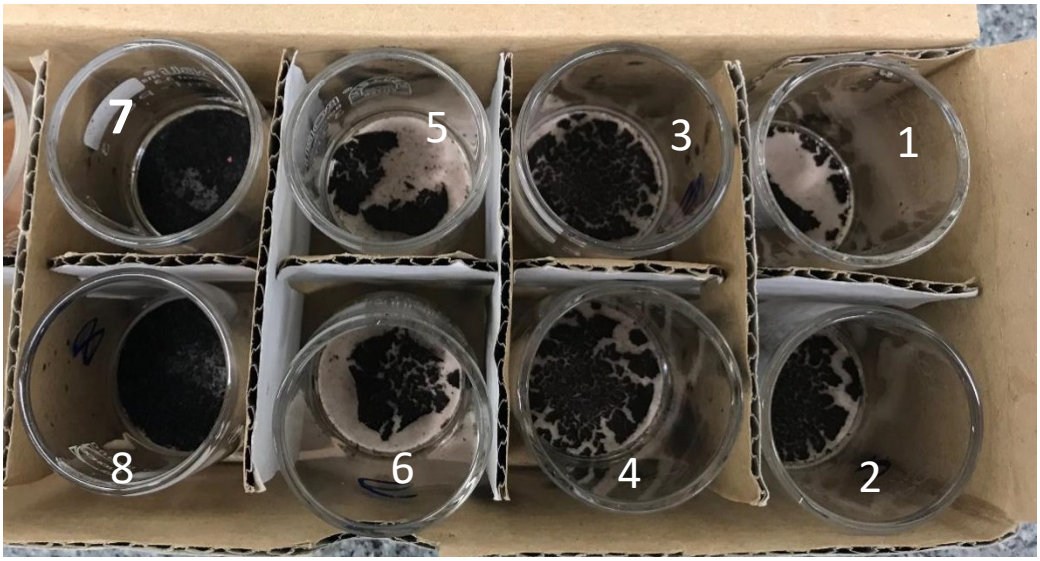

Figure 29. The oven dried hydrolyzed residue after acetone Soxhlet extraction: (1 and 2) the raw spruce inner bark powder; (3 and 4) the liphophilic-free spruce inner bark powder; (5 and 6) the UAE extracted spruce inner bark powder; (7 and 8) the hydrolyzed extractives from raw spruce inner bark powder.

*Table 7. The ^{1}H-NMR chemical shifts for isorhapontin, polydatin and astringin in DMSO-d_6/*pyridine-d_5*. Multiplicity and coupling constants in parenthesis.*

Proton	Isorhapontin δ (ppm)	Polydatin δ (ppm)	Astringin δ (ppm)
H-2’	7.210	7.409	7.000
H-8	7.020	7.020	7.100
H-6’	6.976	7.409	6.832
H-7	6.900	-	6.780
H-5’	6.707	6.707	6.707
H-2	6.806	6.806	-
H-6	6.493	6.493	6.493

H-1''	4.967	4.967	4.967
CH_3O	3.817	-	--
H-2''	3.407	3.439	-
H-4''	3.355	3.227	3.377
H-5''	3.439	3.521	-
H-6''a	3.840	3.869	3.840
H-6''b	3.673	3.673	3.673

*Table 8. The ^{13}C-NMR chemical shifts for isorhapontin, polydatin and astringin in DMSO-d_6/*pyridine-d_5*. Multiplicity and coupling constants in parenthesis.*

Carbon	Isorhapontin δ (ppm)	Polydatin δ (ppm)	Astringin δ (ppm)
C-1	129.307	-	128.992
C-1'	-	-	139.522
C-1''	100.923	-	100.923
C-2	104.975	104.888	104.975
C-2'	110.049	-	113.515
C-2''	73.534	73.080	73.399
C-3	159.098	-	159.073
C-3'	147.949	-	145.670
C-3''	76.799	-	76.674

CH_3O	55.665	-	-
C-4	103.020	102.476	102.928
C-4’	146.907	-	145.891
C-4’’	70.146	69.820	69.972
C-5	158.636	-	-
C-5’	115.699	115.507	115.832
C-5’’	77.319	77.028	77.396
C-6	107.434	107.004	107.298
C-6’	120.365	-	118.805
C-6’’	60.922	60.756	60.922
C-7	125.598	-	125.228
C-8	129.054	128.686	128.992

Table 9. The quantitation of isorhapontin, astringin and polydatin calculated based on ^1H NMR spructrum.

Component	Proton	Area	Molar weight, g/mol	Weight, g	Content, mg/g	In average, mg/g
1,3,5 trioxane	-	0.770	90.078	0.001		
isorhapontin	CH3O	1.073	420.414	0.006	147.169	179.879
	2'	1.551		0.008	212.589	

astringin	6'	3.265	406.387	0.017	432.674	386.651
	5'	2.570		0.014	340.628	
polydatin	3'	2.836	390.388	0.014	361.123	341.993
	4	2.536		0.013	322.864	
908.523						

Table 10. Physical properties of plant oil used in sunscreens.

Raw materials	INCI name	Saponification value	Acid value	Iodine value	Melting point	Density
Jojoba oil	Simmondsia chinensis	92-95	<2	82-88	6.9	-
Sunflower seed oil	Helianthus annulus	188-194	<0.3	125-140	-	0.9
Apricot oil	prunus armeniaca	188-200	0.2-4	97-109	<-10	0.9
Sweet almond oil	Prunus amygdalus	188-197	<2	93-106	-18	0.9
Avocado oil	Peraea gratiaaima	177-198	1.0-7	71-95	8.0	0.9
Olive oil	Olea europaea	180_196	<1	80-88	-	0.9

Table 11. The commercial cost in raw materials of sunscreens (limepop, 2018).

	Composition	Content, 100g	Price, €	Unit Price	
Water phase	Deionized water	50.8	-	-	-
	Glycerin	12.0	0.65	0.07	€/ml
	spruce inner bark	9.2	-	-	-
Oil Phase	Sunflower seed oil	3.0	0.12	0.04	€/ml
	Apricot oil	3.0	0.10	0.03	€/ml
	Sweet almond oil	4.0	0.16	0.04	€/ml
	Avocado oil	3.0	0.14	0.05	€/ml
	Aloe vera oil	2.0	0.08	0.04	€/ml
	Olive oil	2.0	0.25	0.13	€/ml
	Jojoba oil	5.0	0.43	0.09	€/ml
	Wax	4.0	0.13	0.03	€/g
(Surfactant)	Palmitic acid	1.0	1.71	1.71	€/g
(Surfactant)	Stearic acid	1.0	2.84	2.84	€/g
			0.07	€/g	

Table 12. The properties of raw materials

Materials	Properties (Limepop, 2018)
Glycerin	✓ Moisturizing
Sunflower seed oil	✓ Suitable for all skin types ✓ Anti-bacteria ✓ Containing vitamin A and vitamin D and vitamin E ✓ The oil is light and easily absorbed into the skin
Apricot oil	✓ Suitable for sensitive, reddening, scratching and dry skin ✓ Nourishing, softening and moisturizing skin ✓ Anti-aging
Sweet almond oil	✓ Moisturize and nourish skin ✓ Absorbed fast by skin without blocking pores or leaving a greasy residue
Avocado oil	✓ Calm itchy skin, heal chapped skin and replenish dry skin ✓ Hydrate and moisturize skin ✓ Shield skin from ultraviolet radiation ✓ Protect against skin damage
Aloe vera oil	✓ Moisturizer, freshening, healing and calming
Olive oil	✓ SPF 8-10 ✓ Helps other oils absorb to skin ✓ Anti-aging

Jojoba oil	✓ Moisturizer ✓ Help sink to absorb fast ✓ Give lightness to skin
Wax	✓ Make skin soft ✓ Inhibit the moisture evaporation of skin